modulatory factors involved in the establishment of viremia and subsequent infiltration of cytotoxic immune cells is yet well understood. This study thus investigates the role of IRF-2, an attenuator of interferon (IFN) response, on the establishment of viremia and immune cell infiltration during WNV infection.

Methods: Real-time PCR, Western blot, and FACS analyses were used to study the regulation of various IRFs and downstream targets during WNV infection of an astrocytic cell line, A172. Regulation of IRF-2 at the cellular level was then studied using immunofluorescence microscopy. Subsequently, cell lines over-expressing, or with a knockdown of IRF-2 were infected to study the effect(s) that IRF-2 has on WNV production. Finally NK cell assay was performed to investigate the ability of NK cells to lyse these infected cells.

Results: The IRFs 1, 2, 3 and 7 are highly up regulated post-WNV infection. Expectedly, downstream gene targets like IFN-$, IL-12 and IL-6 are up regulated transcriptionally and translationally. In addition, concomitant increased mRNA expression of MHC Class I loading genes like TAP1, TAP2 and $2m with that of HLA-E, translated to an increased surface expression of HLA-E. Interestingly, FACS dot plot analysis revealed that expression of IRF-2 was insufficient to suppress HLA-E expression. Immunofluorescence microscopy further showed the surprising preferential enhanced expression of IRF-2 in the non-infected cells. Finally, infection of IRF-2 over-expressing cells resulted in increased virus production, while a reduction in virus titer was observed in the IRF-2 knockdown cells.

Conclusion: Our results show that IRF-2 is preferentially up regulated in the neighboring non-infected cells, possibly in a homeostatic fashion to regulate pro-inflammatory genes like IFN and cytokines in these cells. On the other hand, the activated activators overwhelm the attenuation effect(s) of IRF-2 in the infected cells. In these infected cells, the inhibitor of NK cell lysis, HLA-E, is expressed. Its expression thus protects the infected cells from the cytotoxic effects of NK cells. Predisposition of neighboring cells to NK cell lysis and/or subsequent infection thus contributes to overall WNV pathogenesis.

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Crimean-Congo hemorrhagic fever virus infects human hepatocytes and induces IL-8 secretion

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Background: Crimean-Congo hemorrhagic fever virus (CCHFV) is a very pathogenic tickborne virus member of the Bunyaviridae family and the Nairovirus genus. The knowledge of CCHFV pathogenesis is improving: recently, new target cells were identified. We and others had demonstrated that CCHFV is able to infect and partially activate monocytes derived dendritic cells and macrophages. During one retrospective study, it was shown that CCHFV was detected in the liver of infected patients.

Methods: Attempting to find other target cells to better understand the pathogenesis of CCHFV, we analyzed the host response induced by CCHFV in hepatocytes infected *in vitro* during a kinetic study.

Results: We noticed that while in HuH7 CCHFV infection elicited a cytopathogenic effect, no visible effect was seen in CCHFV infected HepG2. This intriguing feature led us to analyse the viral parameters expecting a differential cellular response. HuH7 and HepG2 both were shown to be permissive to CCHFV and to replicate the virus at a high load as monitored by plaque titration assay, genomic and anti-genomic strand quantification. The high secretion of IL-8 but no other inflammatory cytokines such as TNF-a, IL-1b indicated that CCHFV induced a response in both hepatocytes. Interestingly, no type I IFN was detected during the kinetic study. In spite of these similarities, we observed a pro-apoptotic CCHF effect more significant in Huh7 than in HepG2 cell lines.

Conclusion: We found that hepatocytes could be considered as CCHFV target cells that could be involved in the pathogenesis disorders. The high IL-8 production by infected hepatocytes associated to the pro-apoptotic effect likely contribute to the disease progress.

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73.004

The course of infection in respiratory infected chickens caused by avian influenza virus A/H5N1

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Background: High pathogenic avian influenza virus (AIV) is an object of research by many scientists in the world. This disease agent is capable of infecting a wide range of varieties of wild and domestic birds. Among the known pathways of any infection the most effective believe airborne and fecal-oral routes. It is believed that chickens infected by the fecal-oral route, i.e. through the gastrointestinal tract. Do not exclude also the aerosol route of transmission of the disease in chickens. In the present study infectious properties of various AIV strains and the degree of sensitivity to this pathogen of respiratory and gastric-intestinal tract of the chickens, as well as the dynamics of dissemination in their body were studied.

Methods: We used eight highly pathogenic AIV A/H5N1, isolated in Russia and CIS countries in chickens that are infected by aerosol, intranasal, intra gastric and oral methods.

Results: All studied AIV strains showed same high virulence for chickens (LD50 is 2—15 EID50) for aerosol challenge. When aerosol challenge sensitivity of these animals to AIV 30 times higher than in the intranasal, 500 times higher than with oral, and 10000 times higher than intra gastric method of infection, indicating a higher susceptibility to AIV of respiratory organs of chickens compared to gastrointestinal tract. Replication of the virus in the membrane of the nasal cavity has already recorded 18 hours after infection (a.i.). The second wave reproduction of the pathogen

observed in many organs and blood serum of chickens and begins a 30 hours a.i. with the highest rates of accumulation of virus in excess of 7 lg EID50/g observed in the lungs, blood serum and kidneys of animals.

Conclusion: According to the results of comparative analysis of LD50 of AIV strains at different options for infections have found that the highest susceptibility to the virus have respiratory organs of chickens compared with the gastrointestinal tract. The primary target organ to AIV in intranasal infected chickens is the mucous membrane of the nasal cavity. In addition to the results of research, we proposed a fecal-nasal transfer mechanism of AIV A/N5N1 in chickens for nature conditions.

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73.005

Role of leukotrienes in resistance and susceptibility to infection by *Histoplasma capsulatum*

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Background: Histoplasma capsulatum (*H. capsulatum*) is a dimorphic pathogenic fungus that causes a wide spectrum of diseases. Macrophages are an important phagocytic cells in host defense against fungi. In order to enhance host defense, these resident cells secrete chemotactic substances such as leukotrienes (LTs) and cytokines that recruit effector cells to the focus of infection. LTs are potent lipid mediators of inflammation and host defense, derived from the 5-lipoxygenase (5-LO) pathway of arachidonic acid (AA) metabolism. We have been shown that the absence of leukotrienes in genetically modified mice (5-LO−/−) or by treating WT animals with pharmacological inhibitor MK886, have increased susceptibility to infection when they are infected with *H. capsulatum*. Recent studies show that susceptibility or resistance of different strains to certain infections, such as *Leishmania amazonensis*, is associated with differential production of LTs. In the present study, we evaluated the production of LTB4 by peritoneal macrophages (PM) from susceptible and resistant mice after challenge with *H. capsulatum* and the effect of LTs in phagocytosis by macrophages of both strains.

Methods: Macrophages from C57BL/6 (susceptible) and sv129 (resistant) mice were infected for 48 h at a ratio of 1:5 (*H. capsulatum*:macrophage). Supernatants were collected and the production of LTB4 was evaluated by ELISA. The phagocytosis was assessed by fluorescence using unopsonized or IgG-opsonized FITC-labeled *H.capsulatum* and MK886, a LTs inhibitor, was added to the cells previously to the infection.

Results: Interestingly, macrophages from resistant mice produced higher levels of LTB4 upon *H. capsulatum* challenge than did those from susceptible mice. As expected, PMs from sv129 phagocytosed 1.9 fold-increased IgG-opsonized-*H.capsulatum* than PMs from C57BL/6. However, phagocytosis of IgG-opsonized-*H.capsulatum* by PMs from C57BL/6 and sv129 are both dependent on endogenous LTs, since when the LTs synthesis is pharmacologically inhibited, the phagocytosis was decreased 10 and 20 fold respectively.

Conclusion: LTs are important mediators involved in the mechanisms of host defense by participating in the patterns of resistance/susceptibility to infection of *H. capsulatum*.

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Different clinical isolates of *Mycobacterium tuberculosis* induced distinctive pulmonary inflammation in mice

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Background: Mycobacterium tuberculosis (*Mtb*) is a virulent intracellular pathogen that infects and persist in host macrophages, resulting in granuloma formation and collagen deposition in the lung. The mechanisms that confer resistance to *Mtb* or results in establishment of disease are poor understood. Data from the literature suggest that differences in *Mtb* virulence contribute to setting up of the disease. In order clarify this aspect, our purpose is to investigate the immune response and lung pathology in mice infected with *Mtb* obtained from distinct clinical isolates. The isolates were recovered from patients with noncavitary (SV 009) or extrapulmonary (SV 068) active tuberculosis.

Methods: Female Balb/c mice were infected intratracheally with 1×105 CFU/100 μL of *Mtb* clinical isolates. Neutrophils and mononuclear cells recruitment to the lung were accessed by bronchoalveolar lavage at 30 days post infection and lung histology were evaluated on 30 and 60 days post infection.

Results: Mice infected with SV 068 showed 22% more neutrophils (9×105 mL) and 70% more mononuclear cells (6×105 mL) recruited to bronchoalveolar space 30 days post infection, when compared with mice infected with SV 009 that presented 5×105 mL of neutrophils and 4.5×105 mL of mononuclear cells. The histology analysis of lung tissue, demonstrated that animals infected with SV 068 present greater number of foamy macrophages containing aggregations of *Mtb*, especially at 60 days post infection. Also, in this period, we observed the presence of more intense infiltrate of neutrophils in perivascular and perialveolar spaces when compared with animals infected with SV 009.